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Magnitude of Hepatitis C Virus Infection in India: Prevalence in Healthy Blood Donors, Acute and Chronic Liver Diseases

Aswini K. Panigrahi,[1] Subrat K. Panda,[1]* Rajesh K. Dixit,[1] Kanury V. S. Rao,[4] Subrat K. Acharya,[2] Srinivasan Dasarathy,[2] and Ambika Nanu[3]

[1]Department of Pathology, All India Institute of Medical Sciences (AIIMS) New Delhi, India
[2]Department of Gastroenterology, All India Institute of Medical Sciences (AIIMS) New Delhi, India
[3]Department of Transfusion Medicine, All India Institute of Medical Sciences (AIIMS), New Delhi, India
[4]Immunology Group, International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India

An enzyme immunoassay (EIA) was developed in-house for the detection of anti-hepatitis C virus (HCV) antibody against the prevailing genotypes in India. The specific reactivity of the test was compared with commercial second and third-generation EIAs and reverse transcription nested polymerase chain reaction (RT-nested PCR). Fifteen thousand nine hundred twenty-two healthy blood donors at the All India Institute of Medical Sciences (AIIMS), New Delhi, India, were screened for anti-HCV antibody. Two hundred ninety-five (1.85%) of these donors were positive. The screening was also used to determine how many patients with acute hepatitis and chronic liver diseases were positive for anti-HCV antibody. Five hundred sixty-four chronic liver disease patients were screened for anti-HCV antibody and 78 (13.83%) were found positive. Two hundred forty-seven sporadic acute viral hepatitis patients were screened for viral infection markers. Hepatitis B and E viruses (HBV and HEV) were the major etiologic agents. HCV was associated with 9% of the acute cases. Anti-HCV core IgM with HCV RNA detection were found to be helpful for the diagnosis of acute HCV infection. ***J. Med. Virol. 51:167–174, 1997.***

KEY WORDS: HCV; anti-HCV prevalence; blood donors; chronic liver disease; acute viral hepatitis; IgM antibody

INTRODUCTION

Hepatitis C virus (HCV) is one of the major causes of posttransfusion non-A, non-B (NANB) hepatitis [Kuo et al., 1989; Choo et al., 1990]. HCV causes acute and chronic hepatitis, liver cirrhosis, and possibly hepatocellular carcinoma. Commercial immunoassays are used routinely to detect anti-HCV antibodies for diagnostic and epidemiological purposes. A clear association between the presence of antibodies to HCV and chronic NANB hepatitis has been demonstrated [Kuo et al., 1989; Aach et al., 1991; Chiba et al., 1991]. The recombinant products of viral genes and synthetic peptides used as antigens are useful for the diagnosis of HCV infection. Second-generation anti-HCV enzyme-linked immunosorbent assays (ELISAs) include antigens from the core, NS3, and NS4 regions. Third-generation anti-HCV ELISAs, which became available recently, include antigens from the NS5 region. The detection of immunoglobulin M (IgM) antibodies against HCV core has been correlated with acute HCV infections, viraemia, and liver histology [Quiroga et al., 1991; Chen et al., 1992, 1995; Clemens et al., 1992]. In the last 7 years, a large volume of information from different parts of the world has accumulated on HCV epidemiology and genotype distribution. However, only limited information on HCV epidemiology and its role in acute and chronic liver diseases is available from the Indian subcontinent [Ramesh et al., 1992; Sood et al., 1992; Panigrahi et al., 1994; Ghuman, 1995]. Two new genotypes of HCV have been described from India [Valliammai et al., 1995; Panigrahi et al., 1996].

This study describes the seroprevalence of HCV in healthy Indian blood donors as well as the magnitude of HCV-related acute and chronic liver diseases in the subcontinent. It also describes the relative value of anti-HCV core IgM antibody for the diagnosis of acute HCV infection.

*Correspondence to: Dr. S.K. Panda, Department of Pathology, AIIMS, Ansari Nagar, New Delhi-110 029, India.
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MATERIALS AND METHODS

Blood Donors

Blood samples were collected from 15,922 healthy blood donors between February 1995 and August 1996 at the Blood Bank, All India Institute of Medical Sciences (AIIMS), New Delhi. Serum samples for testing were separated and stored at 4°C. Screening for anti-HCV antibody was done within 24 hours of collection.

Patients

Between 1992 and 1995, blood samples were collected from 564 patients with chronic liver disease attending the liver clinic of the Department of Gastroenterology, AIIMS. Of the 564 patients, 221 had chronic active hepatitis (male:female = 3.79:1 with a mean age of 35.13 ± 12.23 years) and 343 had cirrhosis (male:female = 3.14:1 with mean age of 40.11 ± 13.3 years). Between 1993 and 1995, blood samples were also collected from 247 clinically proven sporadic acute viral hepatitis patients (male:female = 1.68:1 with mean age of 33.3 ± 12.77 years). Most of these patients were on regular follow up at the Gastroenterology Department, AIIMS. Serum samples were separated from the blood and stored at −20°C. Informed consent was obtained from the patients before inclusion in this study.

Immunoassay

An immunoassay incorporating synthetic peptides from the core, NS3, and NS4 regions was developed in-house for the detection of anti-HCV antibodies. Five peptides corresponding to the amino acid residues 1 to 45 of HCV type 1b, 1 to 45 of HCV type 3g [Panigrahi et al., 1996], 1267 to 1314 of HCV type 1b, 1694 to 1735 of HCV type 1b [5.1.1p, Pawlotsky et al., 1995], and 1920 to 1935 of HCV type 1b [c100p, Pawlotsky et al., 1995] were synthesized on an automated peptide synthesizer (Milligen 9050, Millipore, USA).

The specific reactivity of the individual peptides was evaluated. Twenty-five anti-HCV positive and two anti-HCV negative (by second-generation EIA, UBI, USA) serum samples positive for HCV RNA were screened by individual peptides. ELISA was performed on 96-well microtiter plates (Labsystems, USA) coated with 1 μg of the peptide per well. The peptides were dissolved in 0.01 M phosphate-buffered saline (PBS), pH 7.2, and the plates were coated for 3 hours at 37°C. The untreated sites were blocked with 5% low fat milk powder dissolved in 0.01 M PBS at 37°C for 1 hour. Serum samples at the dilution of 1:10 were incubated at 37°C for 1 hour on the plates. The bound antibodies were detected by incubation with peroxidase-conjugated anti-human IgG, IgM, IgA (DAKO, Denmark) diluted 1:5,000 for 1 hour at 37°C. The plates were developed with orthophenylene diamine (OPD) for 10 minutes at room temperature. The reaction was stopped by the addition of 2N H_2SO_4 and optical density (O.D.) was taken at 492 nm. The cut-off was set at mean O.D. of the known positive and negative serum samples.

For the combined assay, these peptides were mixed at the ratio of 1:1:2:1:1 and coated onto 96-well microtiter plates (Labsystems) at a concentration of 3 μg per well and the tests were carried out as described earlier for the detection of anti-HCV antibody. To determine the cut-off value, serum samples from 30 HCV-infected patients (positive for both anti-HCV antibody and HCV RNA), 30 anti-HCV negative NANB chronic liver disease patients, and 30 anti-HCV negative healthy blood donors were screened by the in-house assay. The distribution of O.D. values was plotted, and the mean O.D. value and the standard deviation were calculated. The cut-off value was determined as described in Results.

For the detection of IgM and IgG antibody to HCV core antigen, two peptides encompassing amino acid residues 1 to 45 of HCV core type 1b and type 3g were used. The microplates (Labsystems) were coated with 1 μg of each peptide per well. The assay was performed as above, except that the bound antibodies were detected by incubation with peroxidase-conjugated anti-human IgM (Sigma, USA) diluted 1:2,000 for 1 hour at 37°C for IgM detection, and peroxidase-conjugated anti-human IgG (Sigma) diluted 1:2,000 was used for IgG detection. The cut-off was set at a mean O.D. of the known positive and negative serum samples.

In case of acute hepatitis, serum samples were screened for hepatitis A virus (HAV) and hepatitis B virus (HBV) infections using micro-ELISA test kits (Organon Technika, The Netherlands), according to the manufacturer's instructions, for the detection of anti-HAV IgM, IgM antibody to hepatitis B core antigen (IgM-anti HBc), hepatitis B surface antigen (HBsAg), and hepatitis B e antigen/antibody (HBeAg/Ab). Hepatitis E virus (HEV) infection was diagnosed by the detection of IgM antibody to ORF-3 using a synthetic peptide-based ELISA developed in-house [Nanda et al., 1995]. The patients negative for either of these IgM antibodies were screened for HCV infection using IgM and IgG detection assays for antibodies to HCV. In case of chronic hepatitis, tests for HBV infection were carried out using HBsAg, HBeAg, and anti-HBe as described above.

The specific reactivity of the in-house assay was compared initially to the anti-HCV antibody detection using a second-generation commercial micro-ELISA assay (UBI). Later, a panel consisting of sera samples from both anti-HCV positive and negative liver disease patients (n = 125) was created from the screening program. These samples were screened for anti-HCV antibody using commercial third-generation EIAs (UBI and Sorin Biomedica, Italy) in duplicate and the in-house assay in triplicate.

The anti-HCV core antibody assay was compared to anti-HCV antibody detection using third-generation commercial EIAs (Sorin Biomedica). Commercial assays were carried out according to the manufacturer's instructions.

TABLE I. Specific Reactivity of the Individual Peptides Used for the Development of an EIA for Detection of Anti-HCV Antibody

S1.NO.	Sample no.	Diagnosis	Anti-HCV (UBI EIA)	HCV RNA	Core	NS3	NS4 (5.1.1p)	NS4 (c100p)
1.	IND054	CAH	+	+	+	+	+	+
2.	IND101	CAH	+	+	+	+	−	+
3.	IND102	CAH	+	+	+	+	+	+
4.	IND103	CAH	+	+	+	−	+	+
5.	IND104	CAH	+	+	+	+	+	+
6.	IND540	CAH	−	+	−	+	−	−
7.	IND674	Cirrhosis	+	+	+	+	−	−
8.	IND823	CAH	+	+	+	−	+	+
9.	IND830	Cirrhosis	+	+	+	+	+	−
10.	IND876	CAH	−	+	+	+	−	−
11.	IND931	CAH	+	+	+	+	+	+
12.	IND1061	Cirrhosis	+	+	+	+	−	+
13.	IND1117	Cirrhosis	+	+	+	+	−	−
14.	IND1132	Cirrhosis	+	+	+	+	+	−
15.	IND1154	Cirrhosis	+	+	+	+	−	−
16.	IND1192	Cirrhosis	+	+	+	+	+	−
17.	IND1326	Cirrhosis	+	+	+	−	+	−
18.	IND1358	CAH	+	+	+	+	+	−
19.	IND1404	CAH	+	+	+	−	+	−
20.	IND1447	Cirrhosis	+	+	+	+	−	−
21.	IND1452	CAH	+	+	+	−	+	+
22.	IND1567	Cirrhosis	+	+	+	+	−	−
23.	IND1751	CAH	+	+	+	+	+	+
24.	IND1905	Cirrhosis	+	+	+	−	−	−
25.	IND1947	Cirrhosis	+	+	+	+	+	+
26.	IND1999	CAH	+	+	+	+	−	−
27.	IND4925	CAH	+	+	−	+	+	−
Total positives			25 (92.6%)	27 (100%)	25 (92.6%)	21 (78.8%)	16 (59.3%)	11 (40.7%)

Reverse Transcription (RT)-Nested Polymerase Chain Reaction (PCR)

RNA was extracted from 100 μl of serum by the acid-guanidinium-thiocyanate method [Chomczynski and Sacchi, 1987] and was screened for HCV RNA by RT-nested PCR, as described earlier [Panigrahi et al., 1994]. All the chronic liver disease patients who showed discrepancy for the presence of anti-HCV antibody using in-house and commercial EIAs were evaluated for the presence of HCV RNA using RT-nested PCR. In addition, 35 patients who were positive for anti-HCV antibody and 45 anti-HCV negative patients using in-house, UBI, and Sorin EIAs (third generation) were evaluated for HCV RNA by RT-nested PCR. All the acute hepatitis patients who were positive for anti-HCV core IgM, IgG, or anti-HCV antibody by third-generation commercial EIAs (n = 16) and 20 acute hepatitis patients who were negative for all serological markers were investigated for the presence of HCV RNA.

RESULTS

The specific reactivity of the individual peptides in detection of anti-HCV antibody was compared to a commercial second-generation EIA (UBI). Sera samples from 25 anti-HCV positive and 2 anti-HCV negative NANB chronic hepatitis patients, which were positive for HCV RNA, were screened by individual peptides. All the samples were reactive with one or more of the peptides (Table I). Twenty-five of the 27 (92.6%) samples were antibody positive against the core peptides, whereas only 18 of 27 (66.6%) had antibodies against the NS4 peptides. The anti-NS3 antibody was detected in 21 (78%) samples.

An EIA was developed incorporating these peptides. Thirty HCV-infected patients (positive for both anti-HCV antibody using a commercial assay and HCV RNA using RT-nested PCR), 30 anti-HCV negative NANB liver disease patients, and 30 anti-HCV negative healthy blood donors were screened using the in-house EIA. The O.D. values were plotted on a scatter plot (Fig. 1). The O.D. values of positive samples varied from 0.733 to 2.045 with a mean value of 1.406 ± 0.37. While the O.D. values of anti-HCV negative liver disease patients varied from 0.11 to 0.388 with a mean of 0.22 ± 0.069, those of healthy blood donors varied from 0.065 to 0.312 with a mean of 0.18 ± 0.066. Negative and positive controls were prepared by pooling anti-HCV negative healthy donor sera and anti-HCV positive patient sera, respectively. These controls were included in all further assays. The cut-off was set at three times the O.D. value of the negative control.

The in-house EIA was first compared with a second-generation commercial EIA (UBI) to determine the specific performance and sensitivity in detection of anti-HCV antibody. Of the 118 anti-HCV positive chronic

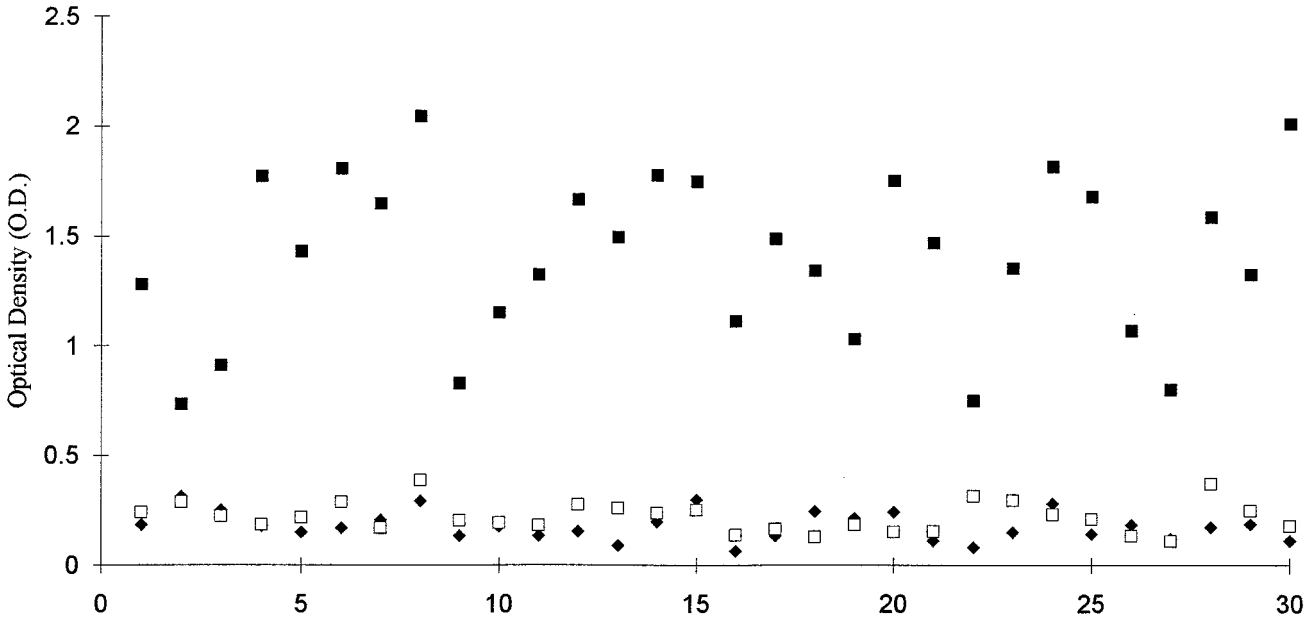

Fig. 1. Distribution of O.D. values of anti-HCV positive patients (■), anti-HCV negative patients (□) and anti-HCV negative healthy blood donors (♦) using the in-house EIA.

hepatitis patients, 116 (98.3%) were positive by the in-house assay (Table II). The two patients who were negative by the in-house assay were also negative for HCV RNA by RT-nested PCR. We detected anti-HCV antibody in an additional 4 of 220 (1.8%) NANB chronic liver disease serum samples by the in-house assay which were negative for HCV antibody by commercial assay (Table II). Three of these four samples were positive for the presence of HCV RNA by RT-nested PCR. Five chronic liver disease patients who were positive for HCV RNA by RT-nested PCR and for anti-HCV antibody by RIBA II, but negative by second-generation commercial EIA were positive for anti-HCV antibody using the in-house assay (data not shown). These findings demonstrated that the in-house assay was highly specific and more sensitive than the second-generation commercial EIA (UBI).

Later, a panel was created including anti-HCV positive and anti-HCV negative liver disease patients (n = 125) from the ongoing screening program in the laboratory. These patients were screened for anti-HCV antibody using in-house EIA and two third-generation commercial EIAs (UBI and Sorin). The results are Tabulated in Table III. Of the 125 patients, 65 were positive for anti-HCV antibody using all three tests and 54 were negative. The discrepancy was observed in six cases. One was positive for anti-HCV antibody by both UBI and Sorin assays, three were positive by the Sorin assay only, and two were positive by the in-house assay only. Of these six patients, only one (16.7%) had HCV RNA and that patient was positive for anti-HCV antibody by the in-house assay only. Further investigation for the presence of HCV RNA was carried out in 35 of the 65 anti-HCV positive and 45 of the 54 anti-HCV

TABLE II. Specific Reactivity of the In-House EIA in Detection of Anti-HCV Antibody

Disease Category	Reactive with second-generation commercial EIA (UBI)	Repeat reactive with in-house EIA
Anti-HCV positive NANB chronic liver disease	118	116 (98.3%)
Anti-HCV negative NANB chronic liver disease	0/220	4/220 (1.8%)
Anti-HCV positive acute viral hepatitis	6	6 (100%)
Postransfusion hepatitis	3/7 (42.9%)	3/7 (42.9%)

negative patients. Twenty six of the 35 (74.3%) anti-HCV positive patients and 3 of 45 (6.7%) anti-HCV negative patients (by all the three tests) were positive for the presence of HCV RNA. On comparing anti-HCV positivity with HCV RNA detection, it was observed that 27 of 37 (72.8%) anti-HCV positive patients by the in-house assay, 26 of 36 (72.2%) by the UBI assay, and 26 of 39 (66.7%) by Sorin EIA had HCV RNA (Table III). This demonstrates that the Sorin EIA, though highly sensitive, may be less specific than the other two tests. The in-house assay compares to third-generation UBI EIA in terms of sensitivity and specificity.

The in-house EIA was used to screen blood donors at the AIIMS blood bank for anti-HCV antibody. Between February 1995 and August 1996, 15,922 healthy blood donors were screened for HCV markers using the in-house EIA. We detected anti-HCV antibody in 295 (1.85%) donors. For cross evaluation, the first 98 anti-HCV positive samples were tested with a second-

TABLE III. Comparison of In-House EIA With Commercial Third-Generation Assays for the Serodiagnosis of HCV

Group	In-house	UBI	Sorin	Total no.	HCV RNA
I	+	+	+	65	26/35
II	–	+	+	1	0/1
III	–	–	+	3	0/3
IV	+	–	–	2	1/2
V	–	–	–	54	3/45
Total	67 (53.6%)	66 (52.8%)	69 (55.2%)	125	30/86
HCV RNA/anti-HCV positive	27/37 (72.8%)	26/36 (72.2%)	26/39 (66.7%)	27/41 (65.9%)	

generation commercial EIA (UBI) in addition to the in-house assay. Of these, 94 (95.9%) were positive by the commercial EIA.

We used this assay in addition to the second-generation UBI-EIA in a screening program for anti-HCV antibody in chronic liver disease patients at the AIIMS. We detected anti-HCV antibody in 13.83% (78/564) patients (10.3% had only anti-HCV antibody and 3.6% had both anti-HCV antibody along with HBsAg; Table IV). Thirty-one percent of these 564 patients were positive for HBV serology only. However, a large proportion (55.1%) of the chronic liver disease patients were negative for HBV and HCV markers.

Seventy (58 HBsAg positive and 12 HBsAg negative) of the 247 (28.3%) sporadic acute viral hepatitis patients were positive for anti-HBc IgM and were diagnosed as acute hepatitis B. Twenty-nine patients (11.7%) were positive for HBsAg only. Of the 70 patients who were diagnosed as HBV infected, 58 were positive only for HBV infection, 11 for both HBV and HEV infection, and 1 patient had both HAV and HBV infection. Forty nine (19.8%) patients were positive only for HEV infection and 16 (6.5%) patients only for HAV infection. Seven (2.8%) patients had HEV infection along with HAV infection.

One hundred five of 247 (42.5%) sporadic acute viral hepatitis patients were negative for HAV, HBV, and HEV infection markers. Of these non-A, non-B, non-E patients, 72 were further screened for anti-HCV core IgM and IgG antibodies. These 72 patients were also investigated for anti-HCV antibody using a third-generation commercial EIA (Sorin). Serum samples from other non-A, non-B, non-E patients had been completely utilized for other studies and were not available. Patients who were positive by any of these tests were investigated for the presence of HCV RNA in their serum by RT-nested PCR and the results are compared in Table V. Using the in-house assay, anti-HCV core IgM was detected in 9 of 72 (12.5%) patients. Six (66.7%) of the IgM positive patients were also positive for HCV RNA in their serum. Of these, three patients had both IgM and IgG antibodies. Four patients had only anti-HCV core IgG antibodies in the serum, two (50%) of whom were positive for the presence of HCV RNA. Anti-HCV antibody by third-generation commercial EIA was detected in 9 of 72 (12.5%) patients, 6 (66.7%) of whom were positive for the presence of HCV RNA. The three patients who were negative for HCV RNA were also negative for anti-HCV core IgM and IgG antibodies. Two (10%) of the 20 patients negative for all serologic markers for hepatitis A through E were positive for HCV RNA. So at a current estimate, non A through E infection accounts for a third of the acute viral hepatitis cases.

DISCUSSION

Most blood banks in India do not screen for anti-HCV antibody mainly due to the high cost of available commercial tests. No indigenous test system is available in the country to screen for anti-HCV antibodies in the donor. Very few reports are available on the seroprevalence of HCV in blood donors from this country. Studies on a limited number of samples tested for anti-HCV prevalence using commercial assays have shown a seroprevalence of 2.5–4% [Sood et al., 1992; Ghuman, 1995]. Second-generation commercial ELISA has its own limitation in terms of specificity and sensitivity as reported by us earlier [Panigrahi et al., 1994]. This fact and the high cost of the available immunoassays necessitated development of an immunoassay based on the amino acid sequences of the prevalent strains in India. A highly specific and sensitive ELISA was developed by us for the detection of anti-HCV antibodies (Tables II, III). Due to the absence of a "gold standard" for the evaluation of anti-HCV tests, it was difficult to compare the specificity and sensitivity. For the standardization of the in-house assay, the discrepancies with the second and third-generation commercial assays were cross checked with HCV RNA detection using RT-nested PCR.

For the determination of reactivity of the individual peptides included in the assay, 27 NANB chronic liver disease patients who were positive for HCV RNA were studied. Of these, 25 were positive for anti-HCV antibodies by second-generation EIA (UBI) and 2 were negative. Of these two, one had antibody against core and NS3 and one against NS3 antigen only (Table I) as detected by these individual peptides. All 27 samples had antibody against one or more of these peptides. It is important to note that antibodies against the core antigen were most frequently detected (92.6%) followed by that for the NS3 peptide (77.8%) (Table I). Feucht et al. [1995] detected NS3 antibodies in anti-HCV positive patients most frequently using a Western blot assay system developed by them. The discrepancy in the ob-

TABLE IV. Prevalence of Anti-HCV Antibody in Chronic Liver Disease Patients

Disease	HBsAg positive anti-HCV negative	HBsAg positive anti-HCV positive	HBsAg negative anti-HCV positive	HBsAg negative anti-HCV negative
Chronic active hepatitis (n = 221)	70 (31.7%)	7 (3.2%)	22 (10%)	122 (55.2%)
Cirrhosis (n = 343)	105 (30.6%)	13 (3.8%)	36 (10.5%)	189 (55.1%)
Total (n = 564)	175 (31%)	20 (3.6%)	58 (10.3%)	311 (55.1%)

TABLE V. Anti-HCV Core IgM and IgG Detection Using an In-House Assay Compared to Anti-HCV Antibody Detection by Third-Generation EIA and HCV RNA Detection

Serum sample	Anti-HCV core IgM	Anti-HCV core IgG	Anti-HCV antibody (Sorin, Italy)	HCV RNA
1.IND2073	–	–	+	–
2.IND1516	–	+	–	–
3.IND2341	–	+	+	+
4.IND1903	+	–	+	+
5.IND2122	+	+	+	+
6.IND2418	+	–	–	–
7.IND2171	+	–	+	+
8.IND2367	+	+	–	+
9.IND1776	+	–	–	–
10.IND2363	+	–	–	+
11.IND2448	–	+	+	+
12.IND1457	–	+	–	–
13.IND2122	+	+	+	+
14.IND1194	–	–	+	–
15.IND2110	–	–	+	–
16.IND1278	+	–	–	–
Total	9	7	9	8

servation may be due to the difference in the test system and the nature of the antigen used in this study.

The specific reactivity of the in-house assay was compared to one second-generation and two third-generation commercial EIAs. Variations between the assay systems were observed in detection of anti-HCV antibody. The results of this study (Tables II, III) suggest that the in-house EIA is highly specific and more sensitive in detection of anti-HCV antibody than the second-generation commercial EIA and compares to that of third-generation assays. Variations between two third-generation commercial assay systems were also observed (Table III). While it was observed that the third-generation Sorin EIA is highly sensitive, it may not be as specific as the third-generation UBI EIA and our in-house EIA.

Anti-HCV antibodies were detected in 295 of 15,922 (1.85%) blood donors screened in our blood bank during $1\frac{1}{2}$ years. To the best of our knowledge, this is the first extensive report from India on the seroprevalence of HCV in the normal population. The AIIMS is the premier tertiary care hospital in the country. Patients from all over the country are referred to this hospital. Their relatives contribute to a significant proportion of blood donors. Therefore, the results reflect the situation in India with its vast geographical diversity. The seroprevalence rate is considerably higher in comparison to the United States (1.2%), Italy (0.9%), France (0.7%), Germany (0.4%), Taiwan (0.8%), and Japan (1.2%) [Choo et al., 1990]. The carrier rate of human immunodeficiency virus (HIV) is 0.1% and that of (HBV) varies from 2 to 5% in our country. Screening for HBV and HIV is mandatory in all blood banks in India. However, screening for anti-HCV antibodies is yet to be carried out routinely in any of the blood banks. Recipients of anti-HCV positive blood products are at a very high risk of developing posttransfusion hepatitis than recipients of anti-HCV negative blood [Van der Poel et al., 1990]. Anti-HCV positive patients with normal alanine transaminase (ALT) levels should not be considered as "healthy carriers" because they are often viraemic and might represent a large overlooked reservoir of infection [Silini et al., 1995], possibly with inapparent chronic hepatitis. Low-risk healthy blood donors who are positive for anti-HCV antibodies should be informed and followed up with supplementary tests like RIBA, HCV RNA detection, and liver biopsy. This may confirm if the individual is suffering from HCV-related liver damage.

Earlier we reported the magnitude of HCV infection in chronic liver disease patients from India [Ramesh et al., 1992; Panigrahi et al., 1994]. Here we report the seroprevalence of HCV in a larger sample size using both commercial and the in-house assay. We detected anti-HCV antibodies in 13.83% of chronic liver disease patients. This is similar to our earlier observation. This study was carried out on 564 consecutive chronic liver disease patients. Two hundred twenty one (39.2%) of them had chronic active hepatitis whereas 343 (60.8%) had cirrhosis. In this country a large number of patients come to the hospital when they are very sick. So we mostly see patients with advanced disease state. In addition, AIIMS being a tertiary care hospital, we encounter more cirrhosis cases than chronic hepatitis cases. The seroprevalence of anti-HCV antibodies in the population remains almost the same (Table IV).

HBV was observed to be the major cause of sporadic acute hepatitis in India followed by HEV (Fig. 2). However, a large proportion of these patients were negative for HBV, HEV, and HAV infection markers. IgM anti-HCV core is the first marker for active antibody response in most cases of acute HCV infection [Clemens et al., 1992]. We used the most immunogenic portion of the HCV core region [Ishida et al., 1993] to detect IgM and IgG anti-HCV antibodies. In our earlier studies, we observed that multiple genotypes of HCV are prevalent in India and that type 3 variants, including a new subtype 3g, contribute to the majority of HCV infection [Panigrahi et al., 1996]. So, we used peptides derived

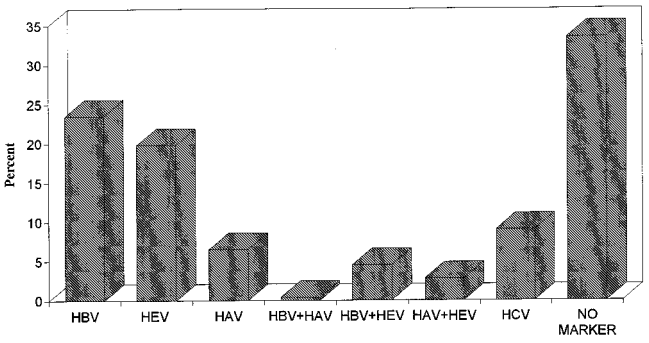

Fig. 2. Etiology of sporadic acute viral hepatitis in India.

from HCV type 1b and 3g sequences for the detection of anti-HCV core antibodies. Using the in-house assay developed with these peptides, we could detect anti-HCV core IgM in 9 of 72 non-A, non-B, non-E hepatitis patients suffering from acute viral hepatitis. IgG could be detected in another four of these patients in the absence of IgM antibody, two of whom were positive for HCV RNA. Nine (4 had anti-core IgM, 2 had only IgG, and 3 were negative for IgM/IgG using the in-house assay) of the 72 patients were positive for anti-HCV antibodies by a third-generation EIA (Sorin). The three patients who were negative for IgM/IgG antibodies were also negative for HCV RNA, suggesting that these may be false positives. Of the nine patients positive for IgM antibodies by the in-house assay, six were positive for HCV RNA. The circulating HCV RNA titre may vary considerably and occasionally fluctuate below the detection limit of the PCR assay during HCV infection [Farci et al., 1991]. This may explain why three of the nine anti-core IgM positive patients were negative for HCV RNA. Appearance of IgM and IgG core usually occurred simultaneously [Clemens et al., 1992], explaining the observation that three patients had both IgM and IgG antibodies. Anti-HCV IgG is detected for a long time, but is unrelated to the course of infection. Only IgG positivity may suggest progression to the chronic state or to acute exacerbation on a chronic HCV infection.

It was observed that 16 of the 72 (22.2%) non-A, non-B, non-E acute viral hepatitis patients had antibodies against HCV if we take into consideration the results of all three tests, i.e., anti-HCV IgM, anti-HCV IgG, and third-generation commercial EIA. Eight of these 16 (50%) patients were HCV RNA positive. In addition to the HCV antibody positive patient, investigation for HCV RNA was done in 20 of these (72 – 16 = 56) patients who were negative for HCV antibody. HCV RNA was detected in 2 (10%) of them. Considering all the anti-HCV core IgM positive patients (9 of 72, 12.5%), an additional 10% of the patients who were positive for HCV RNA (seronegative for hepatitis A through E viruses) had acute HCV infection; it can be extrapolated that the HCV is associated with 9% of the total number of acute viral hepatitis cases in India.

These observations suggest that detection of anti-HCV core IgM is a more sensitive assay than is the third-generation commercial EIA for the diagnosis of acute HCV infection. In addition, HCV RNA assay increases the detection level of acute HCV infection. As HCV infection frequently becomes chronic, markers for the diagnosis of acute infection is necessary. Acute hepatitis and acute exacerbation of chronic hepatitis due to HCV infection need to be dissected in patients positive for both IgM and IgG antibodies. The answer may lie in the detection of serum IgM anti-HCV in the absence of IgG antibody and histopathologic analysis.

The new and yet-to-be-identified GBV-C/HGV viruses may play a significant role in both acute and chronic hepatitis cases in India. In our recent studies, HGV appears to be associated with at least 10% of chronic and fulminant hepatitis cases (results not shown). Further studies are in progress.

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